

Figure 1

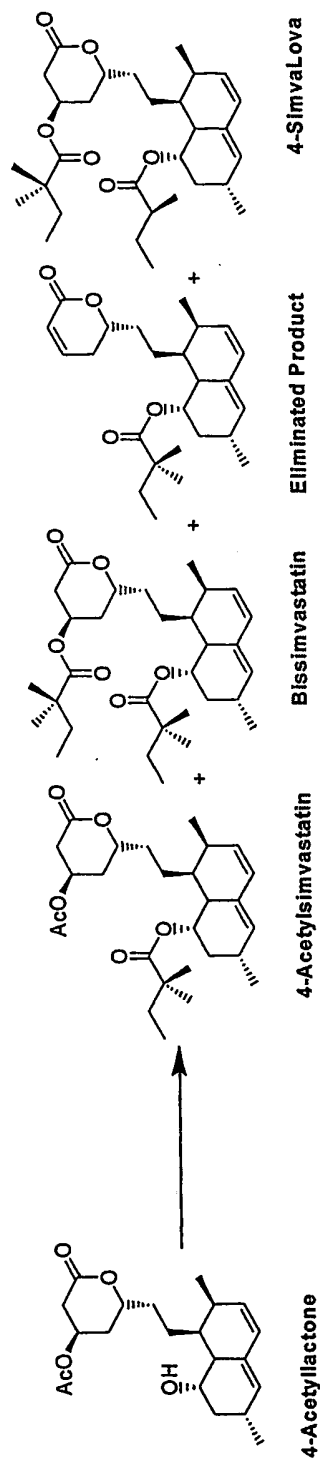


Figure 2

Table 3. Survey of conditions for the acylation of 4-acetylactone

Run	BF ₃ .OEt ₂ mol%	DCM:MeCN	Time h	Mass ¹ balance	4-Ac Sim	SM %	DiOAc %	4-Ac Lova	Elimin %	4-Sim- Lova	BisSim ³ %
1	8	1:1	0.5	94.8	90.8	1.2	2.8	1.1	0.5	2.7	0.9
2	8	1:1	5.3	85.3	90.7	0.6	3.3	1.2	0.9	2.5	0.8
3	(Old) 8	9:1	6.5	89.7	91.1	0.6	2.7	1.1	0.8	2.7	1.0
4	4	1:1	17.5	78.9	36.3	55.9	3.9	1.0	1.3	1.0	0.6
5	8	5:1	1.1	92.8	96.9	0.3	0.9	0.5	0.4		1.0
6	8	5:1	4.5	87.1	96.1	0.5	1.0	0.5	0.7		1.2
	(Stock)										

¹Crude weight yield. ²Lovastatin with 2,2-dimethylbutyrate at the 4-position. ³2,2-Dimethylbutyrate at the 4,8-positions.

Figure 3

Table 4. Purification of 4-Acetylsimvastatin by precipitation from MeOH

	DCE:MeCN	Wt yield %	4-AcSim		SM		DIOA		4-Ac Lova		Elimi		BisSim	
			%		%		%	c	%		n	%	%	
Crude product	5:1	112 ¹	96.4		1.0		0.9		0.3		0.7		0.7	
After precipitation		93	98.5		0.2		0.9		0.2		0.2			

¹ Contaminated with 2,2-dimethylbutyric acid

Figure 4

Table 8. Isolation of Simvastatin

Run	Scale g	Batch	Theoret Yield g	Isolated Yield g	Yield %	Acid %	Simva %	4-Acsim %	Elimin %	Lova %
1	50	Pooled: various	45.4	33.8	74.4	0.2	96.7	1.7	1.2	0.2
2	45	Pooled same batch	40.9	27.7	67.7	0.3	97.8	1.3	0.3	0.4
3	25	Pooled Same batch	22.7	17.2	75.7	0.3	97.7	1.3	0.3	0.4
4	96.6	Single batch	87.8	73.4	83.6	0.7	97.5	1.0	0.6	0.2

Figure 5

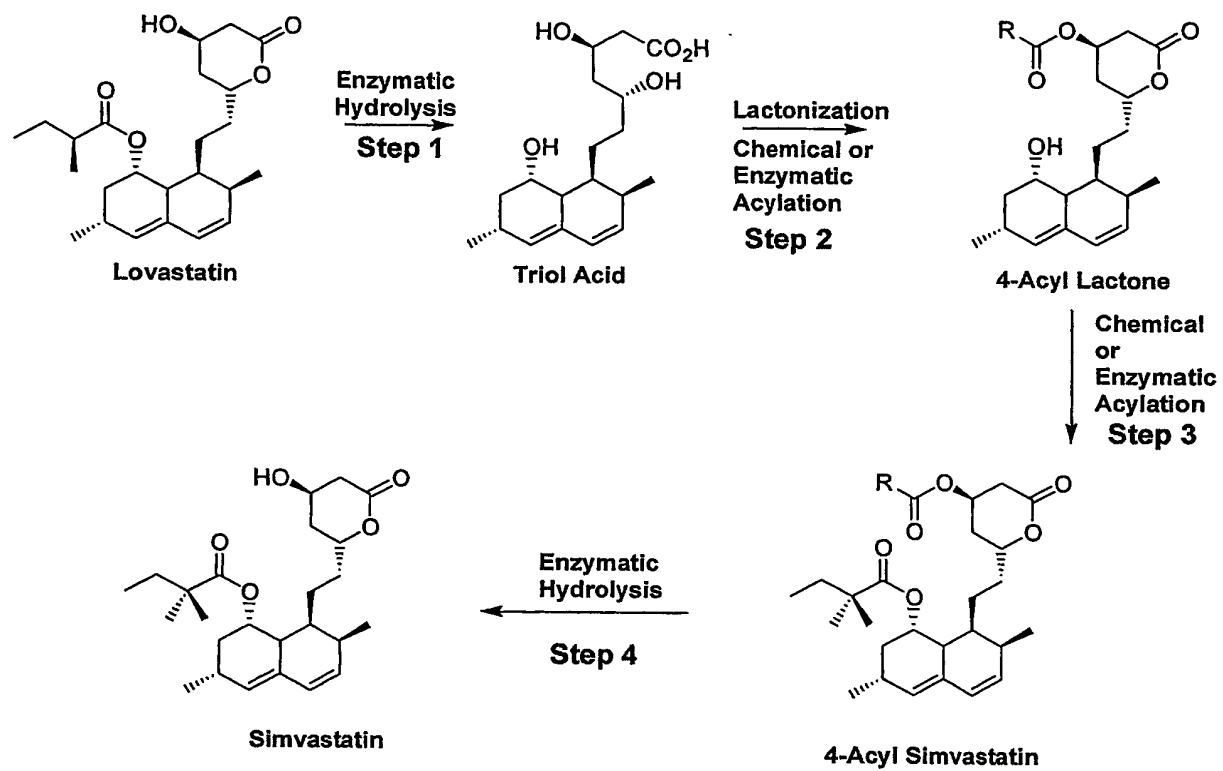


Figure 6

Figure 6A

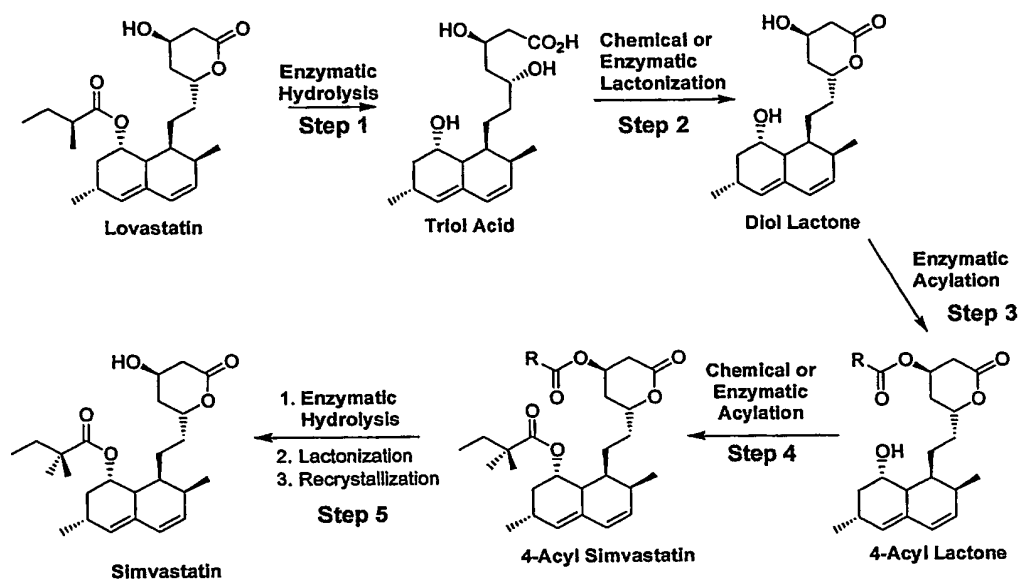


Figure 6B

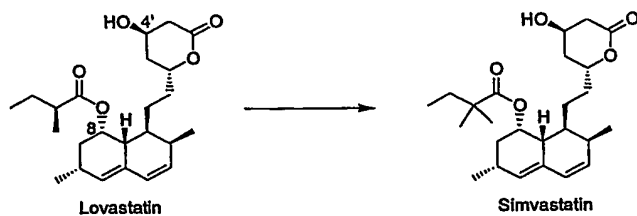


Figure 7

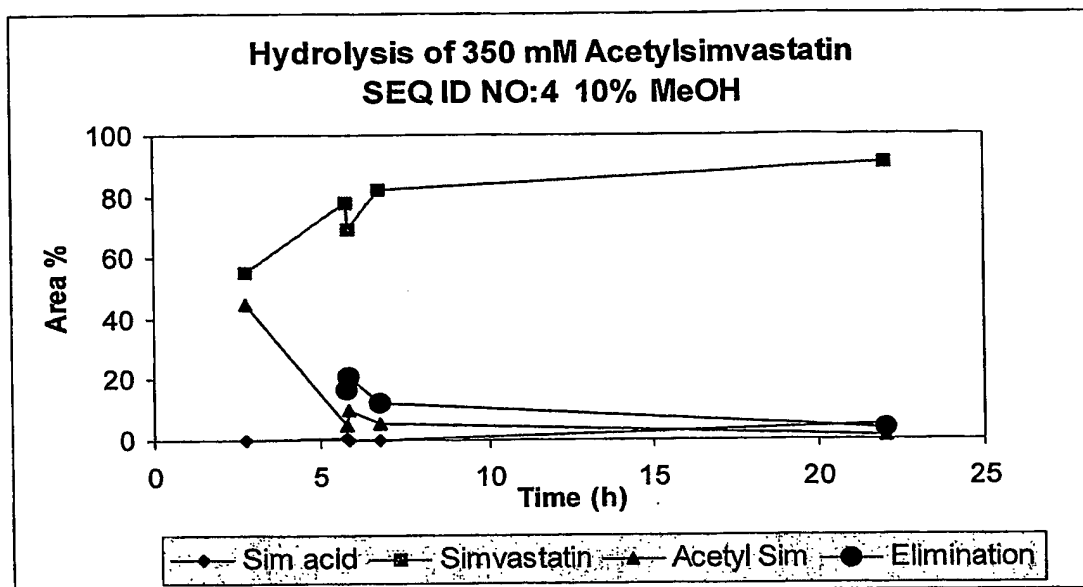


Figure 8

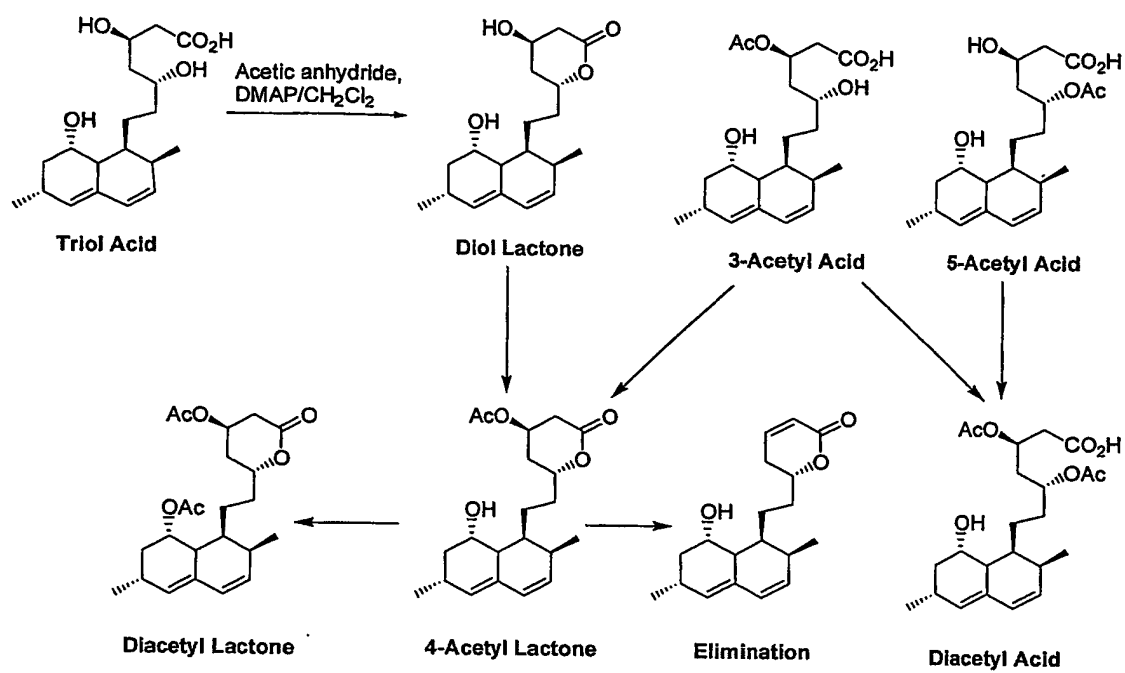


Figure 9

Figure 9A

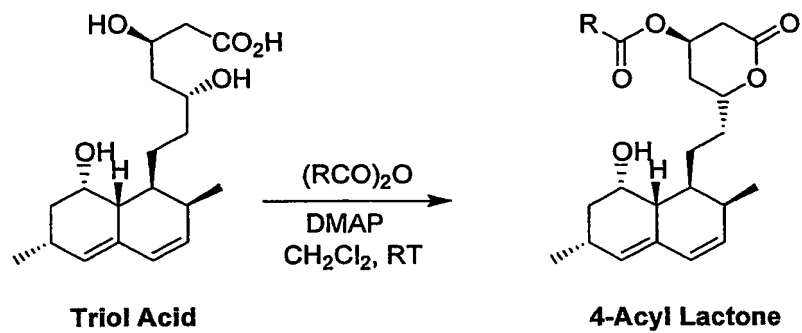


Figure 9B

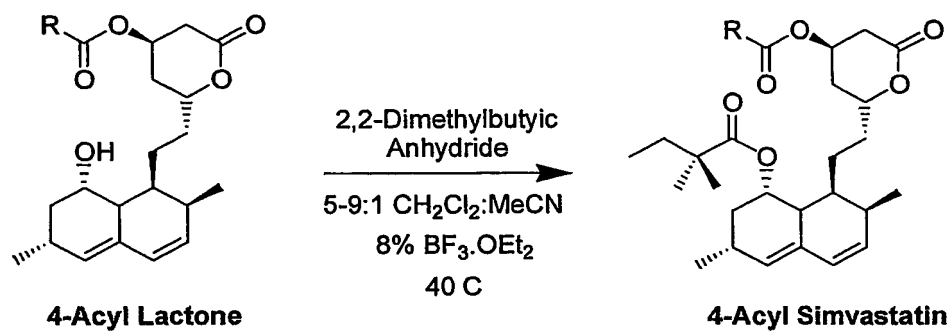


Figure 9C

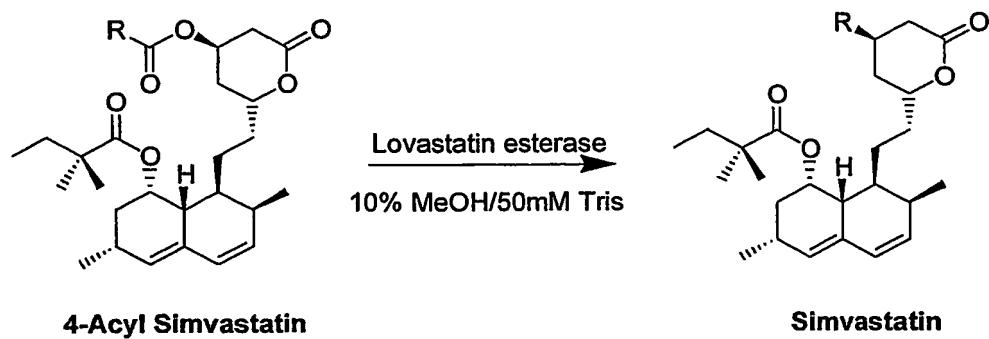


Figure 10

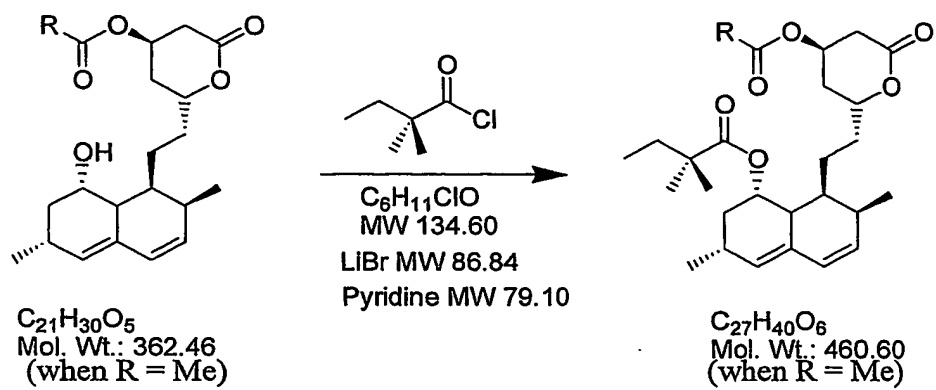


Figure 11

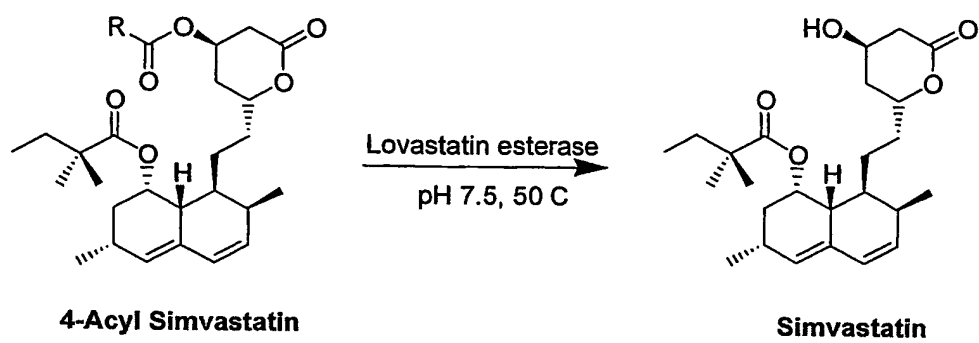


Figure 12

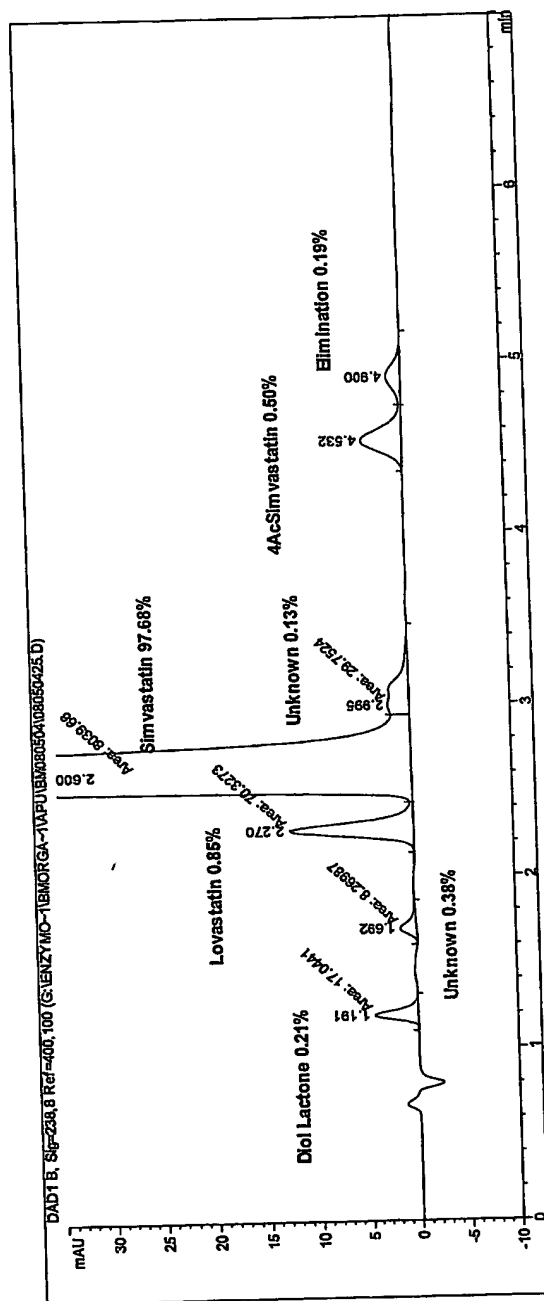


Figure 13

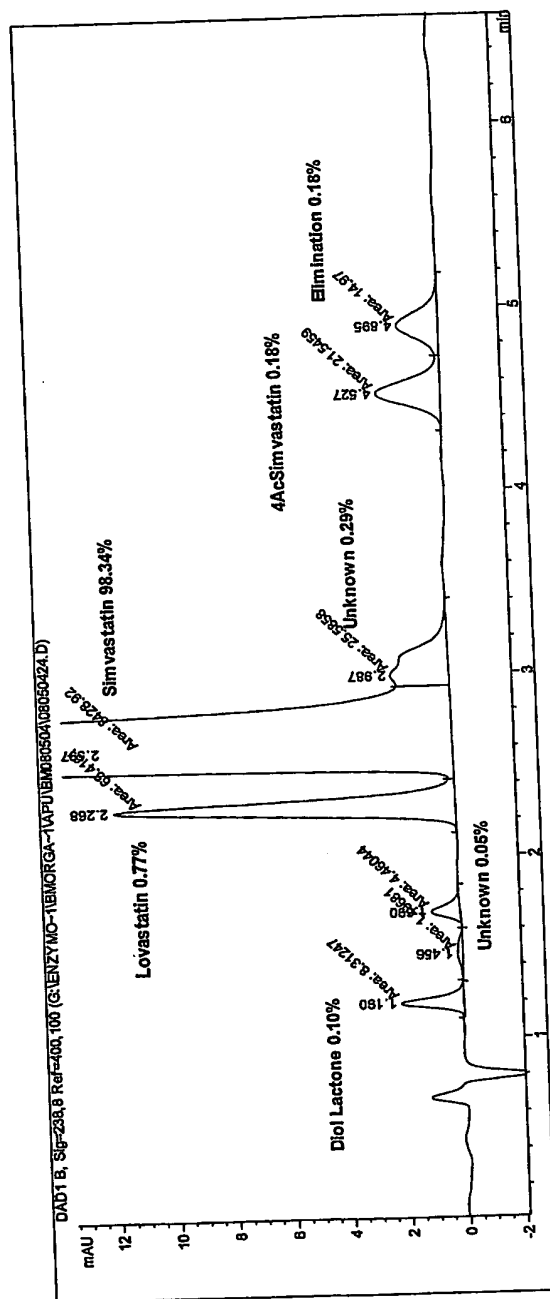


Figure 14

Run	Starting Material	Diol lactone (%)	Diacetyl lactone (%)	Elimination or Diacetyl Acid (%)	4-Acetyl Lactone (%)	Yield %	Mass Balance (%)
1	Triol acid	0.5	1.7	5.1	91.1	87.4	94.2
2	Triol acid	0.5	1.7	6.4	91.0	83.2	93.0
3	Triol acid	2.3	1.3	8.1	88.3	88.8	100.9
4	Triol acid	2.4	1.3	8.1	88.2	90.9	103.1

Conditions: DMAP 15 mol%; Anhydride: 3 eq; 0°-RT, CH₂Cl₂

Figure 15

Figure 15A

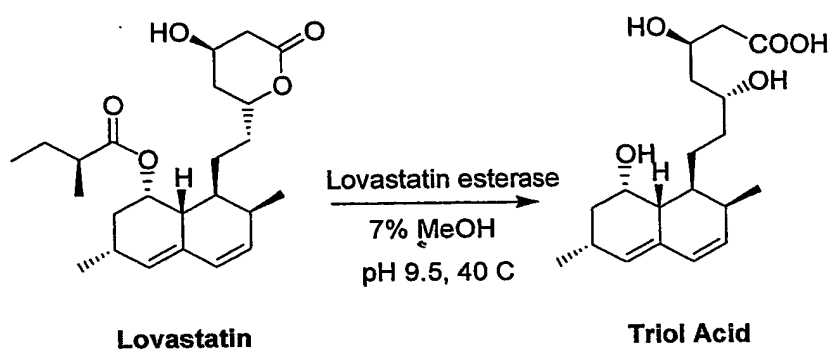


Figure 15B

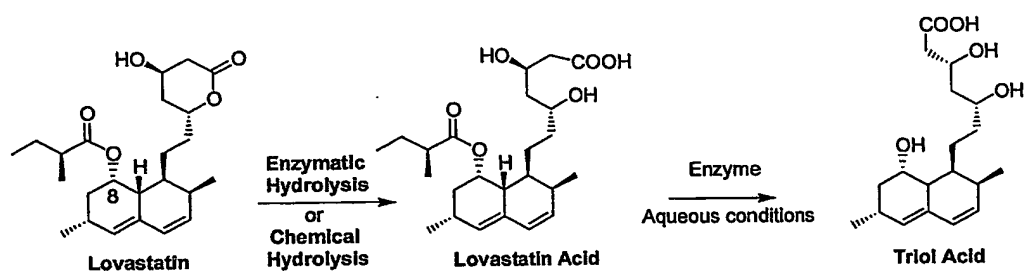


Figure 16

Figure 16A

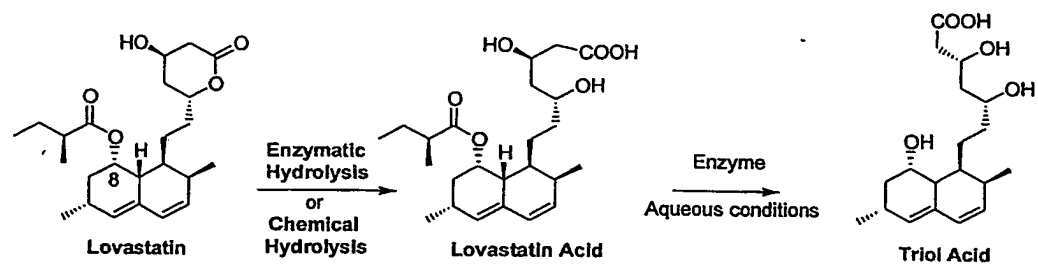


Figure 16B

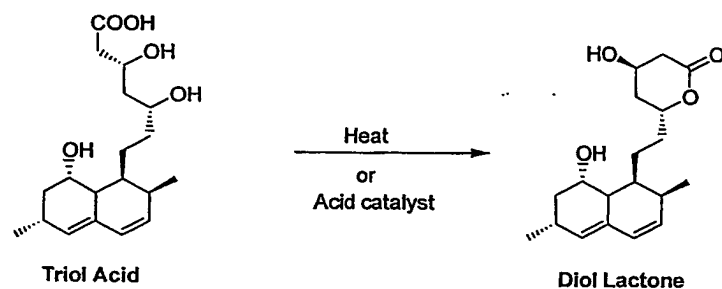


Figure 16C

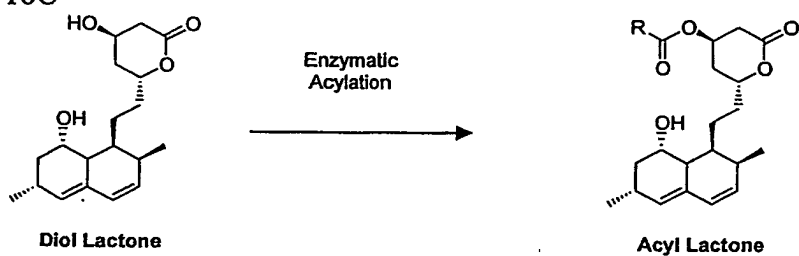


Figure 16D

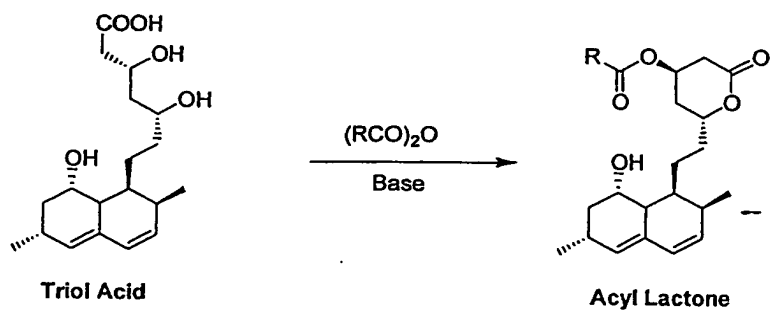


Figure 16

Figure 16E

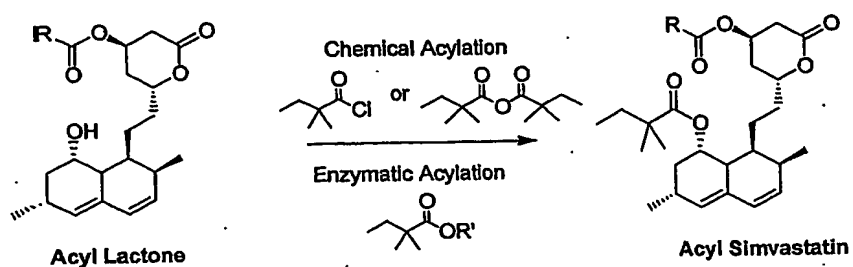


Figure 16F

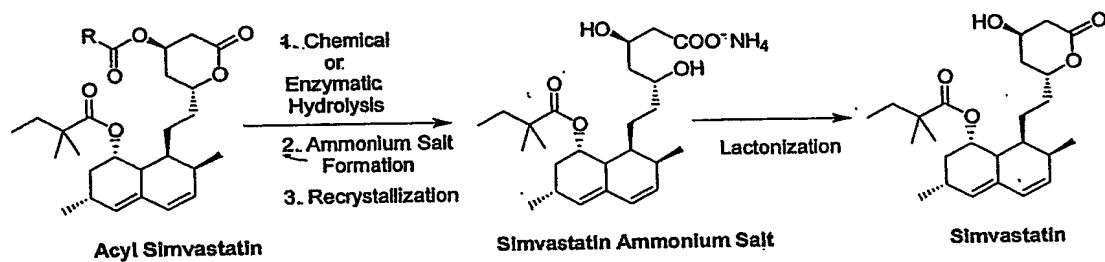


Figure 17

Figure 17A

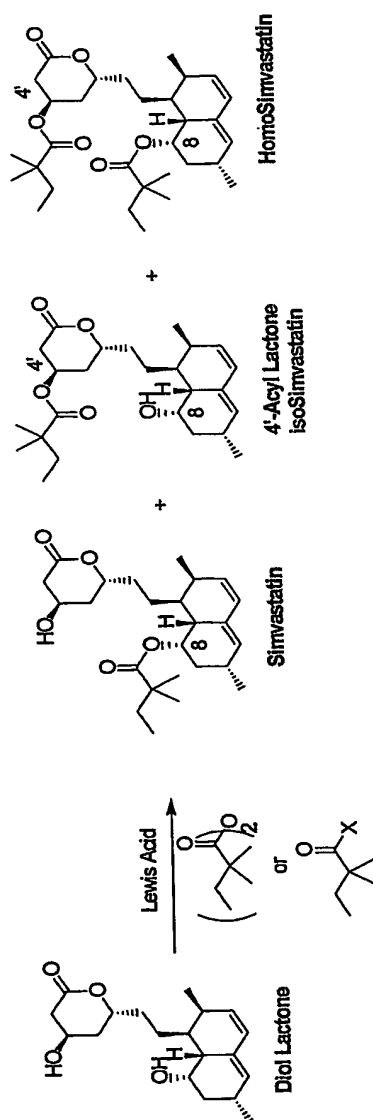


Figure 17B

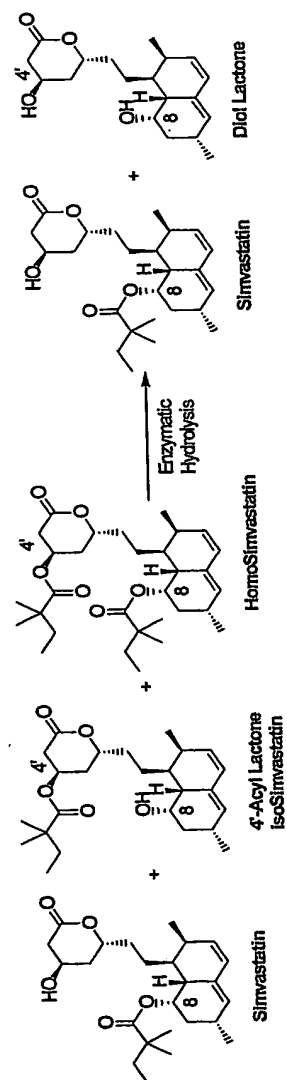


Figure 18

Figure 18A

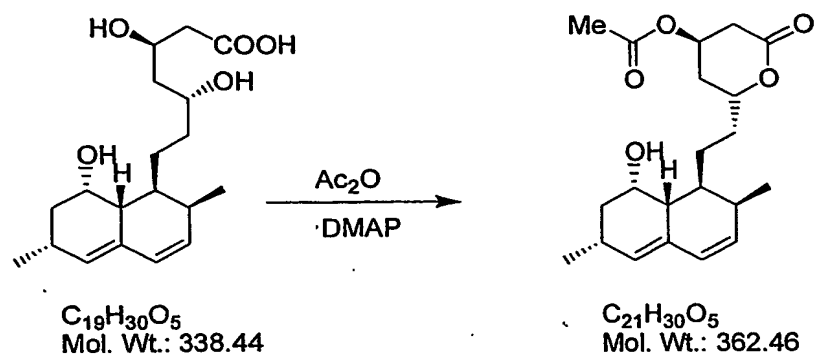


Figure 18B

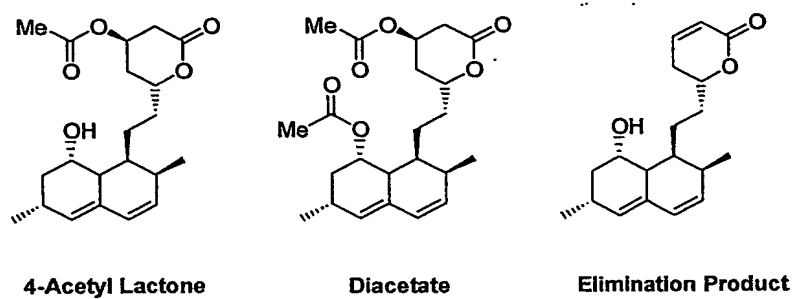


Figure 18

Figure 18C

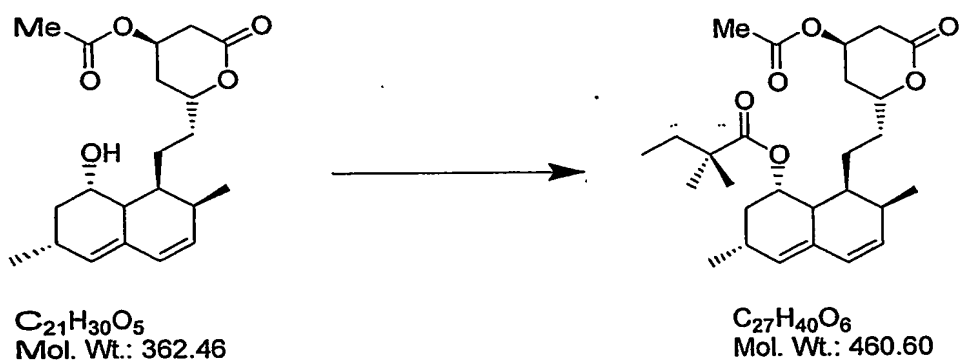


Figure 18D

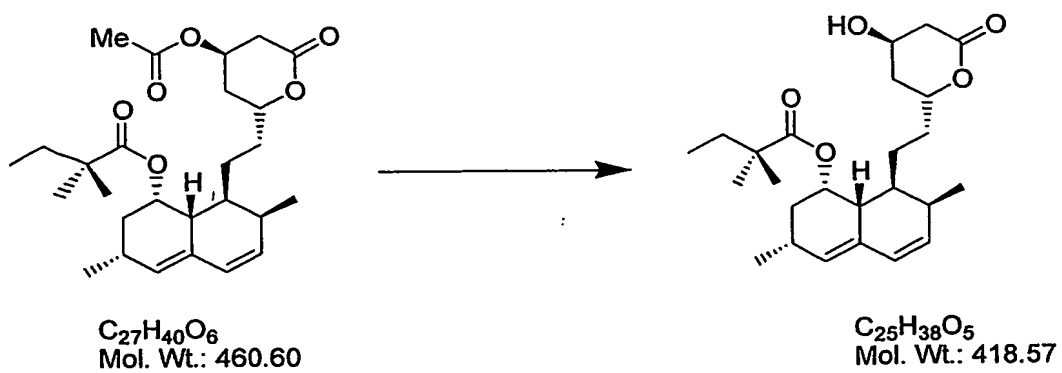


Figure 18

Figure 18E

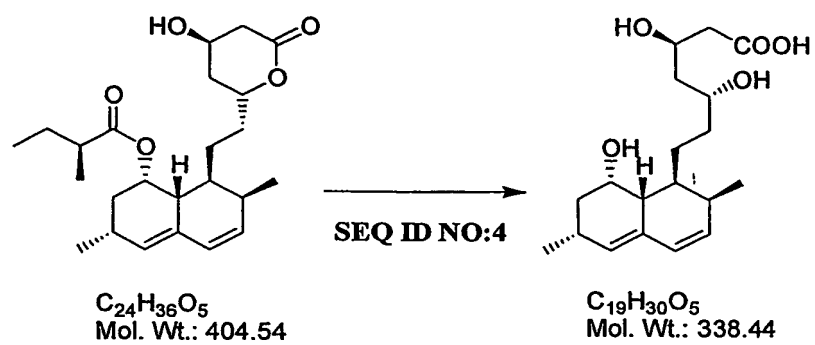


Figure 19

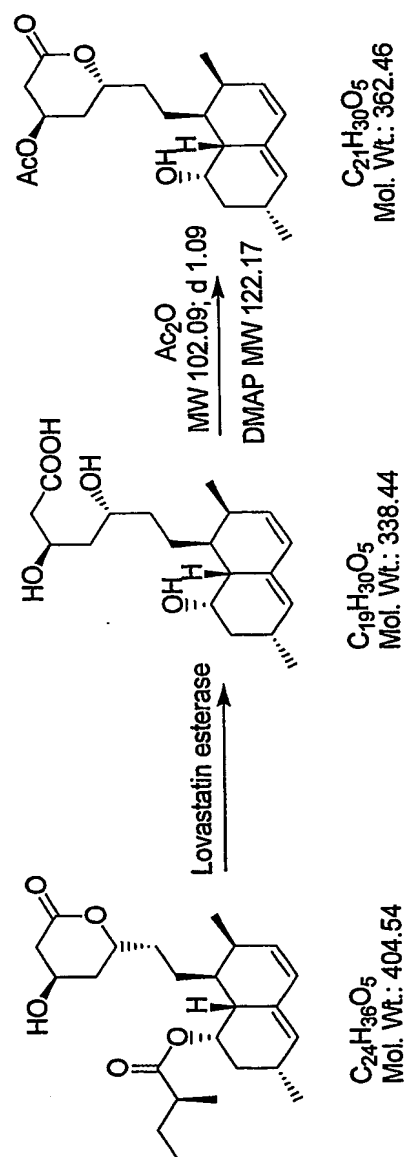


Figure 20

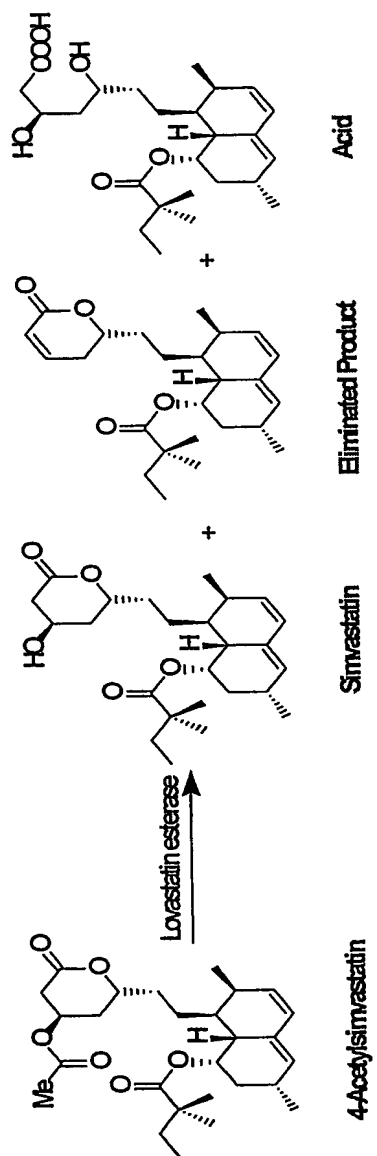


Figure 21

	Triol Acid	Diol Lactone	1.66	Lovast atin	Simvas tatin	2.85	AcLov	AcSimv	Elimi	HPLC Assay %	CHN
M-31		0.11		0.34	99.5						✓
2645-82/83	0.47	0.15	0.08	0.31	97.0	0.47		0.92	0.19	84.5	X
2645-84		0.96	0.73	0.48	95.2	0.39		0.93	1.35	86.3	✓
ML 38 g		0.43	0.62	0.45	96.8	0.55	0.25	0.52	0.36	93.5	n/d

Figure 22

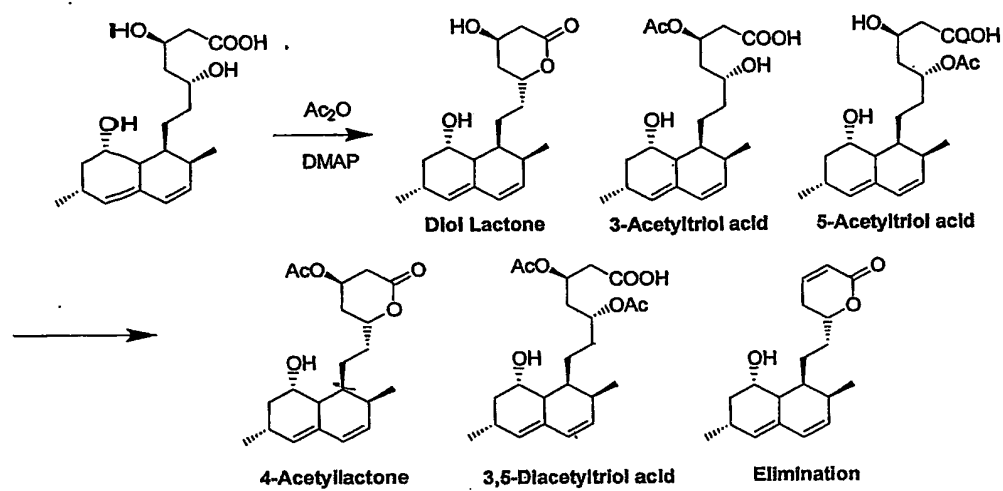


Figure 23

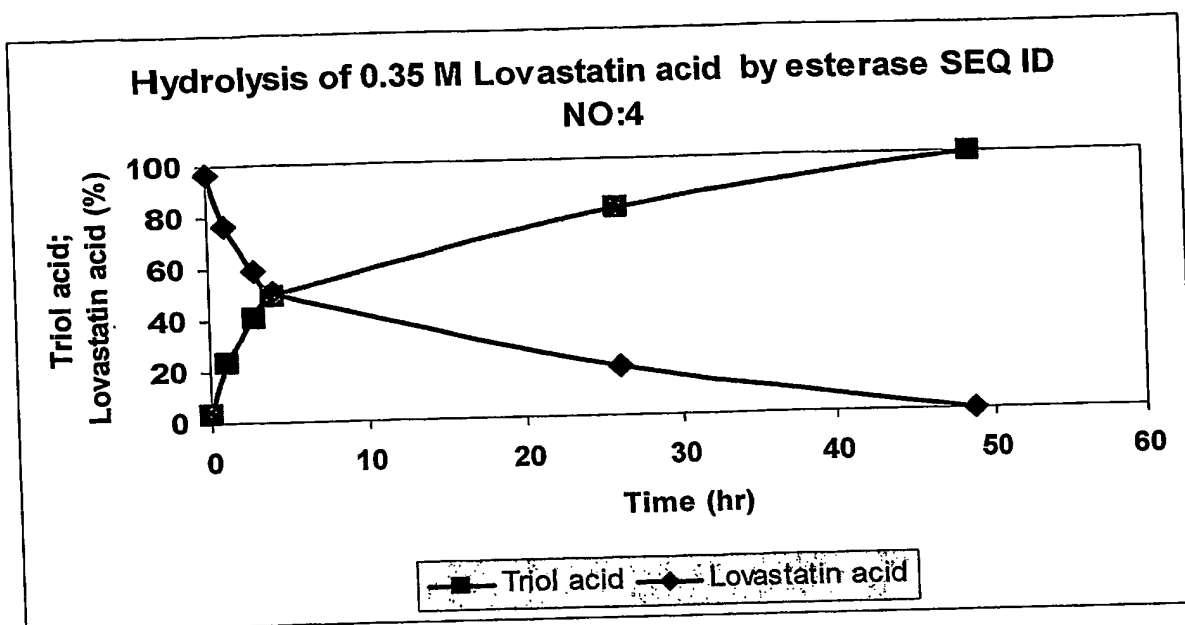
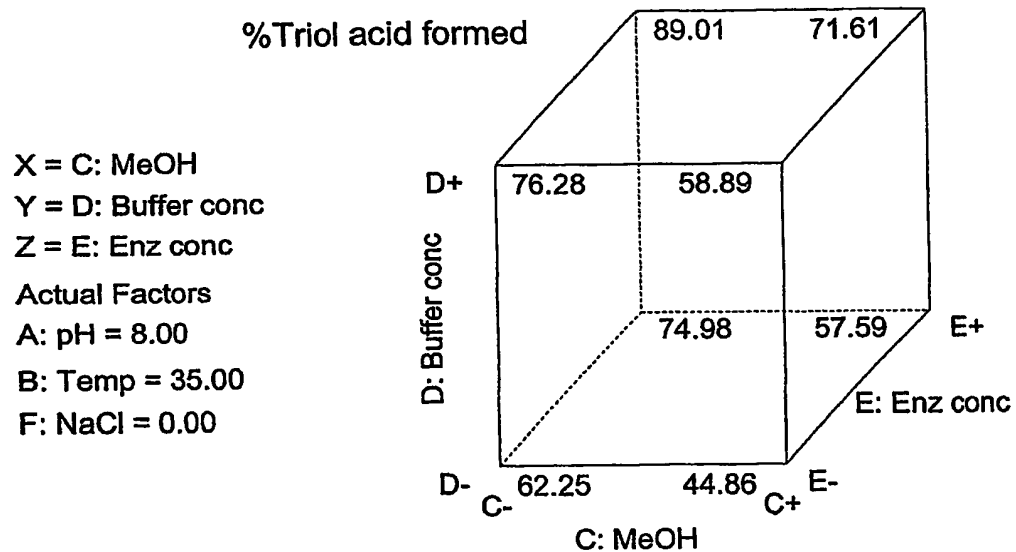


Figure 24

Run no	Block	Parameter levels							Responses	
		pH	Temp °C	MeOH %	Tris-HCl mM	Enzyme mUMB U ⁻¹	NaCl mM	Rate ²	Conversion ³	
1	Block 1	+1	-1	+1	-1	-1	+1	3.69	45.07	
2	Block 1	+1	+1	+1	-1	+1	-1	2.15	41.35	
3	Block 1	-1	-1	+1	+1	+1	+1	4.62	52.33	
4	Block 1	-1	+1	+1	+1	-1	-1	5.43	54.22	
5	Block 1	+1	-1	-1	+1	+1	-1	14.84	94.86	
6	Block 1	-1	+1	-1	-1	+1	+1	13.89	65.62	
7	Block 1	-1	-1	-1	-1	-1	-1	5.43	63.11	
8	Block 1	+1	+1	-1	+1	-1	+1	13.27	54.38	
9	Block 2	+1	-1	-1	-1	+1	+1	19.48	60.37	
10	Block 2	-1	+1	+1	-1	-1	+1	4.90	32.87	
11	Block 2	-1	-1	-1	+1	-1	+1	6.86	59.52	
12	Block 2	-1	-1	+1	-1	+1	-1	6.46	59.84	
13	Block 2	-1	+1	-1	+1	+1	-1	20.75	98.06	
14	Block 2	+1	+1	+1	+1	+1	+1	11.22	66.58	
15	Block 2	+1	+1	-1	-1	-1	-1	12.38	61.76	
16	Block 2	+1	-1	+1	+1	-1	-1	4.66	64.27	

Figure 25



Parameter	Level	Effect on triol acid production
MeOH	High	Negative
Tris-HCl conc.	High	Positive
Enzyme conc.	High	Positive
NaCl conc.	High	Negative
pH		No significant effect
Temperature		No significant effect

Figure 26

Run no	Block	Parameter levels			Responses	
		Methanol %	Tris-HCl mM	Enzyme mUMB U ¹	Rate ²	Conversion ³
1	Block 1	-1	50	-1	6.58	85.00
2	Block 1	+1	200	-1	4.50	62.52
3	Block 1	0	125	0	7.71	88.23
4	Block 1	+1	50	+1	7.55	87.55
5	Block 1	-1	200	+1	11.30	98.23
6	Block 1	+1	125	0	7.40	88.24
7	Block 2	-1	50	+1	11.88	99.60
8	Block 2	+1	125	0	7.53	88.85
9	Block 2	-1	200	-1	6.84	84.19
10	Block 2	0	200	+1	7.73	88.84
11	Block 2	0	50	-1	5.33	73.40
12	Block 2	+1	125	0	8.20	93.89
13	Block 3	+1	125	0	7.18	87.79
14	Block 3	+1	20	0	7.30	89.24
15	Block 3	+1	230	0	7.73	90.75
16	Block 3	+1.4	125	0	5.64	76.20
17	Block 3	+1	125	1.4	10.43	98.03
18	Block 3	+1	125	0	7.35	89.94
19	Block 3	-1.4	125	0	10.33	98.20
20	Block 3	+1	125	-1.4	4.53	68.85

Figure 27

DESIGN-EXPERT Plot

total conv
X = A: methanol
Y = C: enzyme

Actual Factor

B: Tris-HCl = 125.00

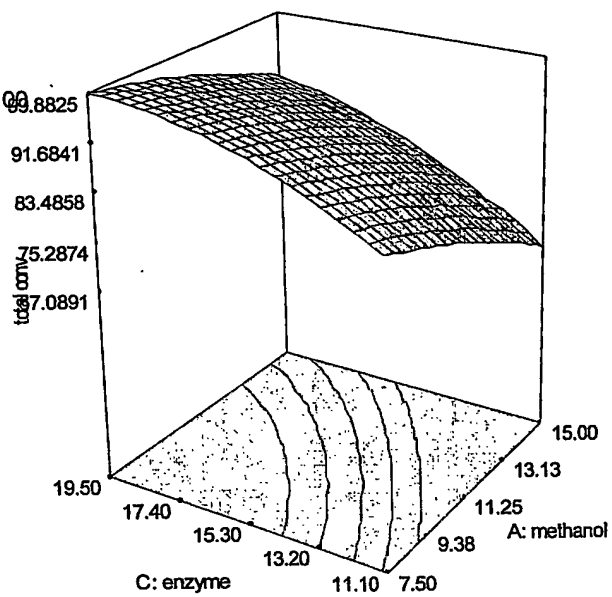


Figure 28

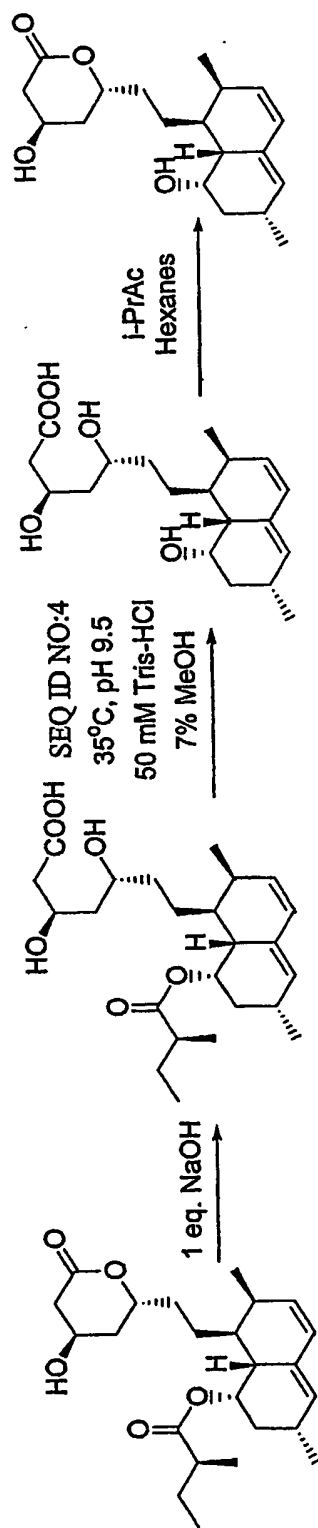


Figure 29

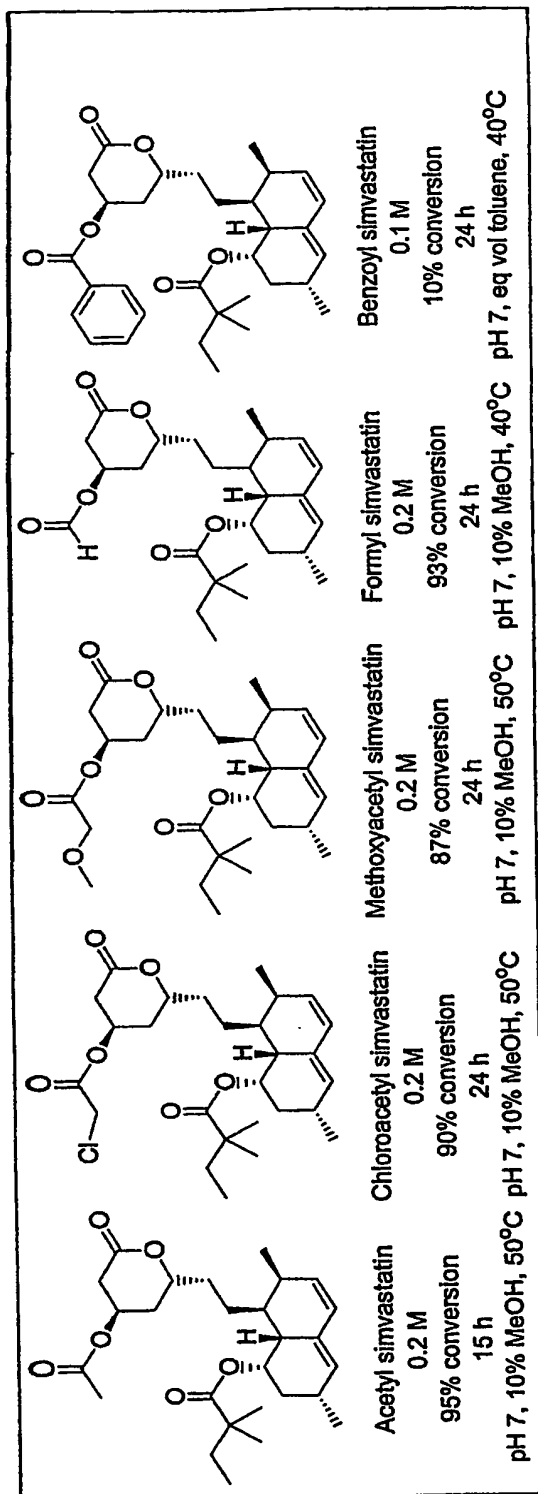


Figure 30

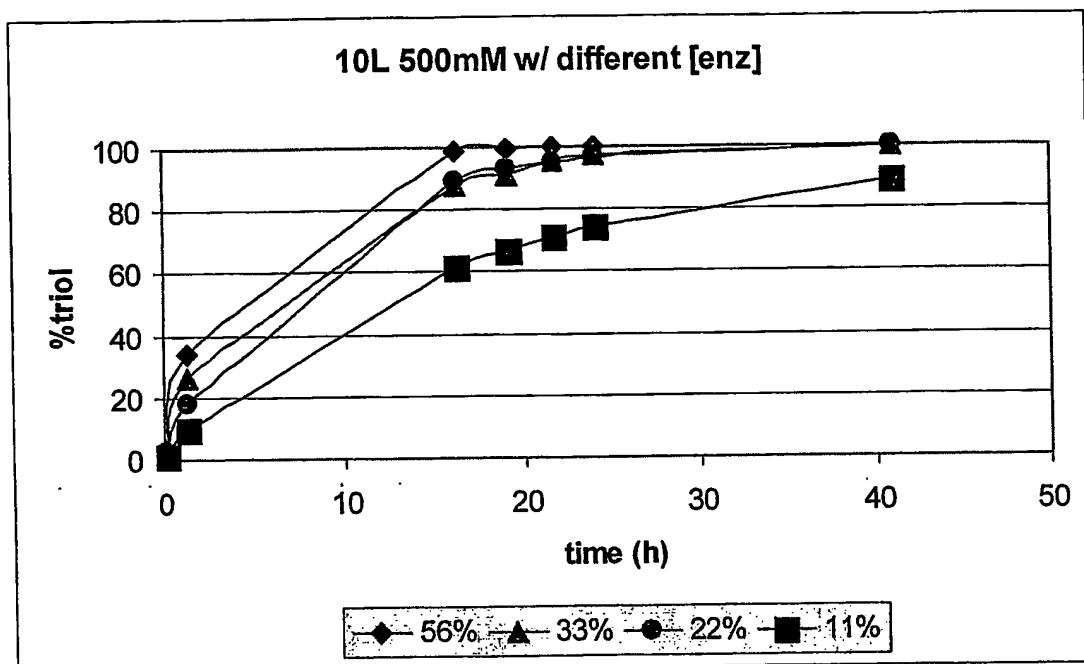
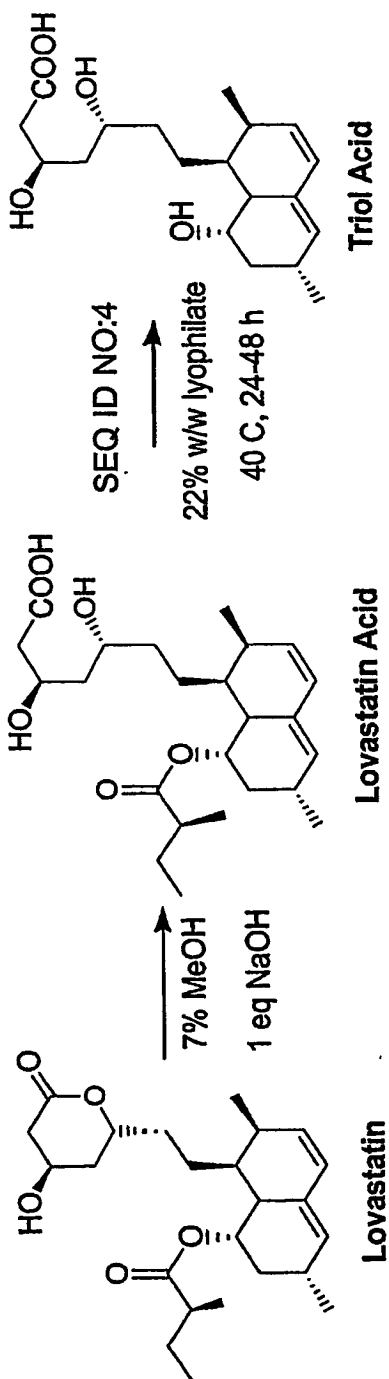


Figure 31



Substrate g	Lyophilate g	Time h	Conversion %
50	11.3	28	99
100	22.6	28	97.5
		42	99.4
100	22.6	28	97.8
		44	>99

Substrate 500 mM; 7% MeOH; 40 C

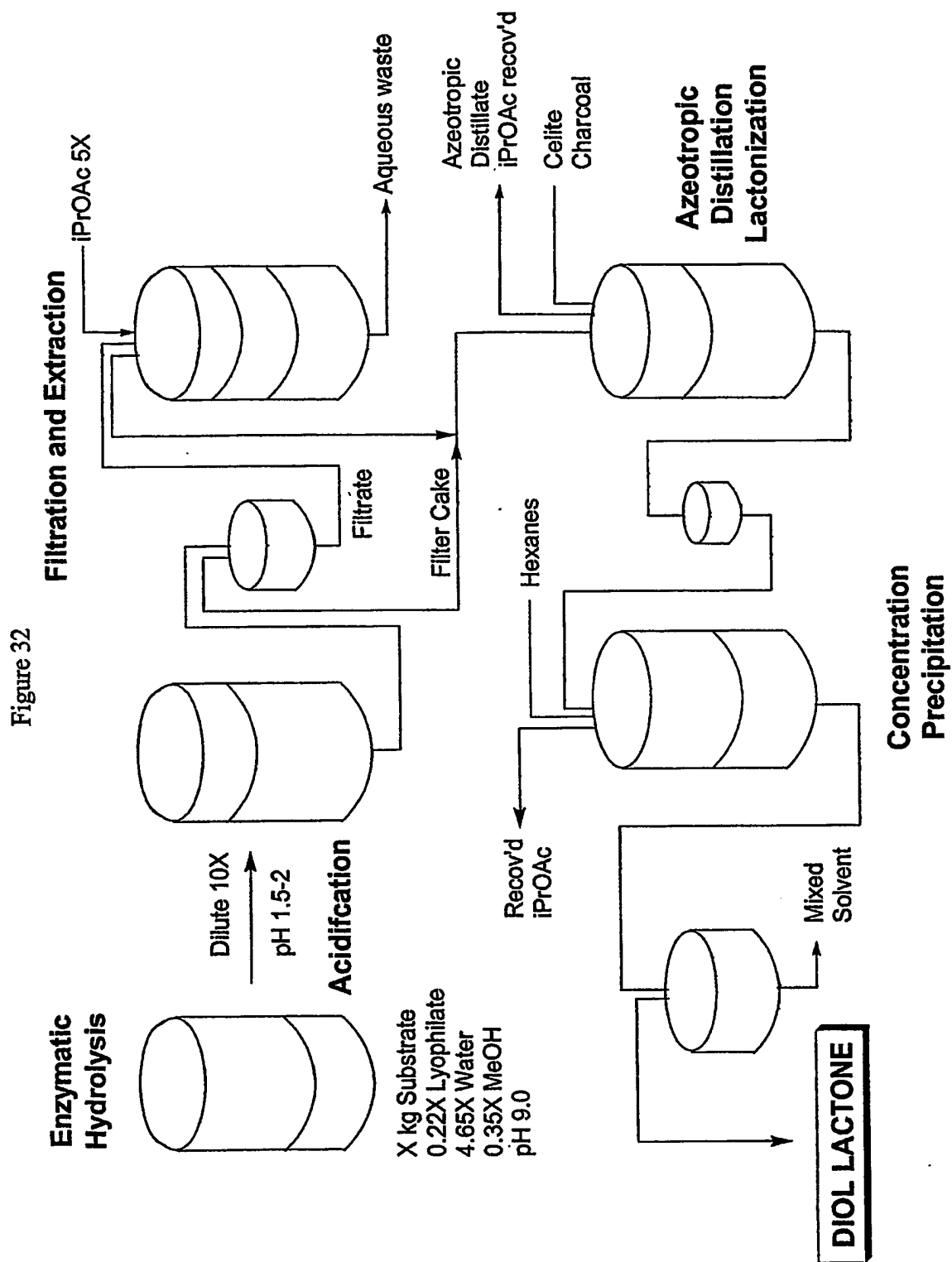
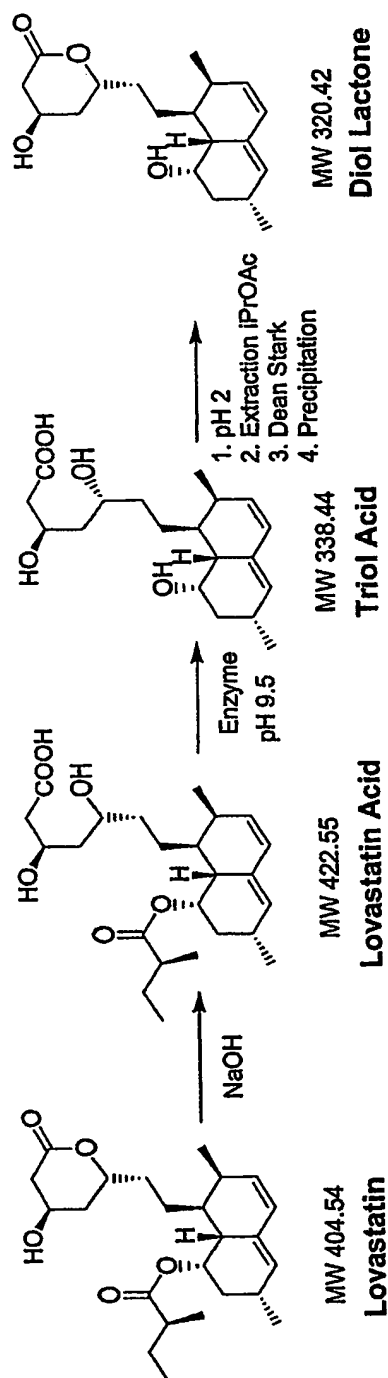


Figure 33



Reactions: Scale/mM	Workup	Theoretical	Product	Yield	
		Yield	g	%	
		g			
350 mM pooled	Continuous Liquid/Liquid Dean Stark	5	4.19	83.8	90% accounted
12 g 500 mM	Centrifugation MeOH	9.58	7.85 Two crops	81.9	91.9% accounted
10.8 g 500 mM	Warm extraction Centrifugation	8.62	6.63	76.9	85% accounted
50 g 500 mM	Centrifugation	39.6	39.2 evaporated	99	0.6 g in washes
100 g 500 mM	Filtration	79.2	61.6	77.8	90.8% accounted

Figure 34

Figure 34A

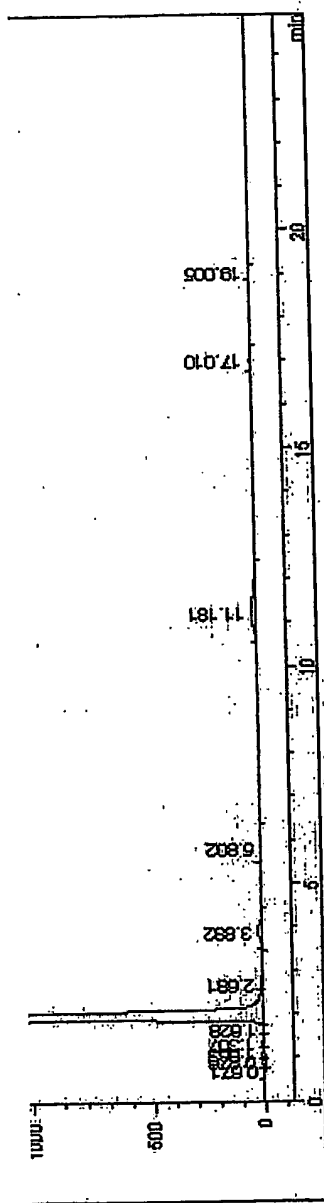


Figure 34B

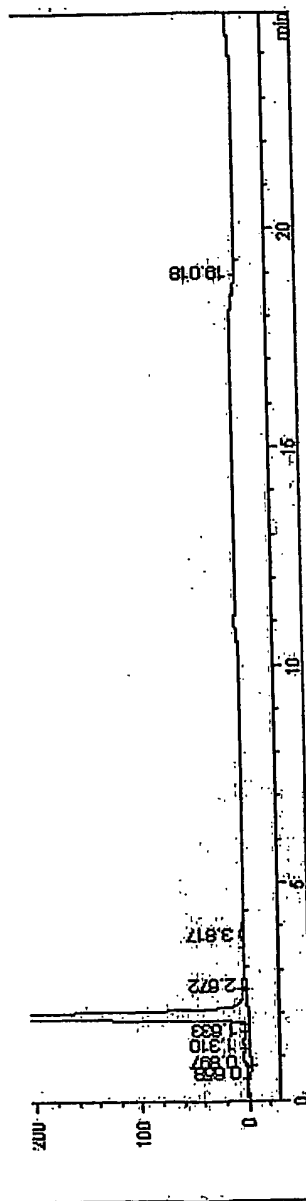


Figure 35

Figure 35A

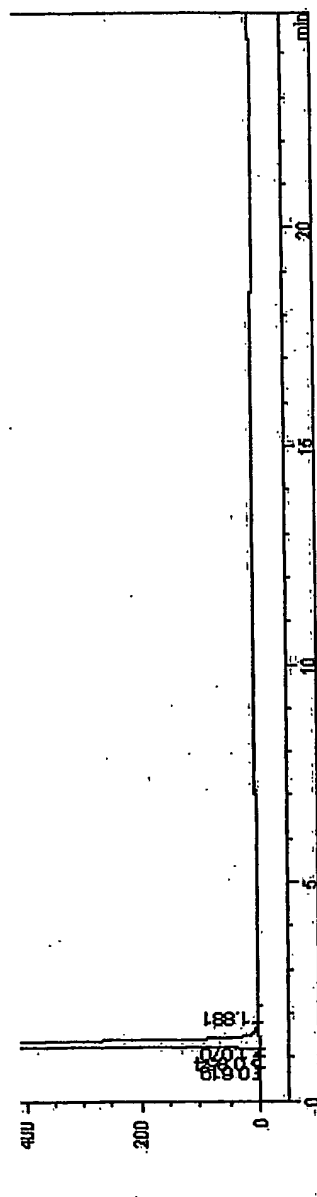


Figure 35B

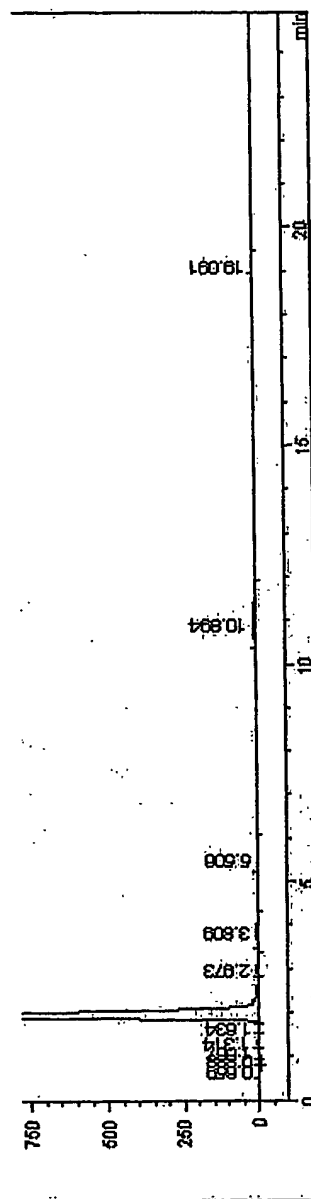


Figure 36

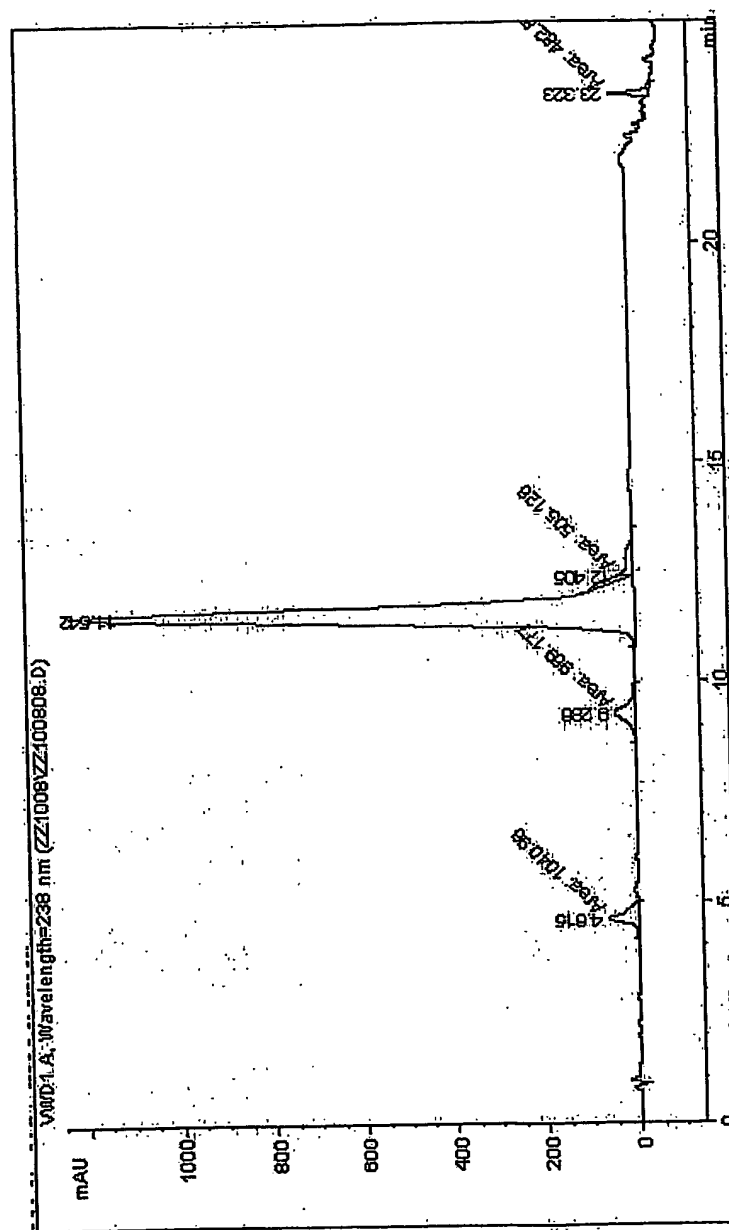


Figure 37

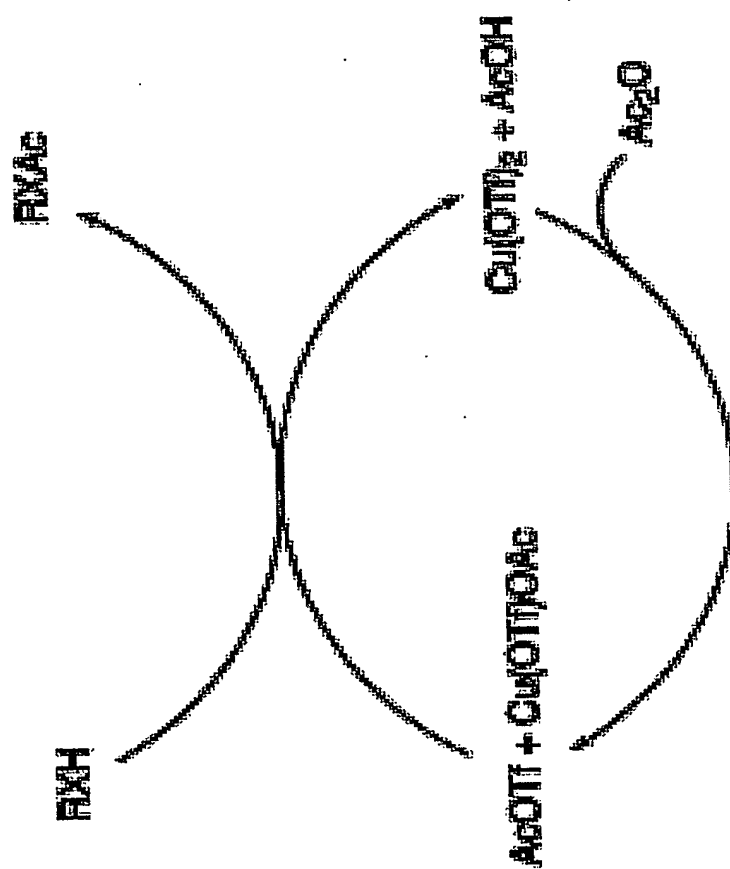


Figure 38

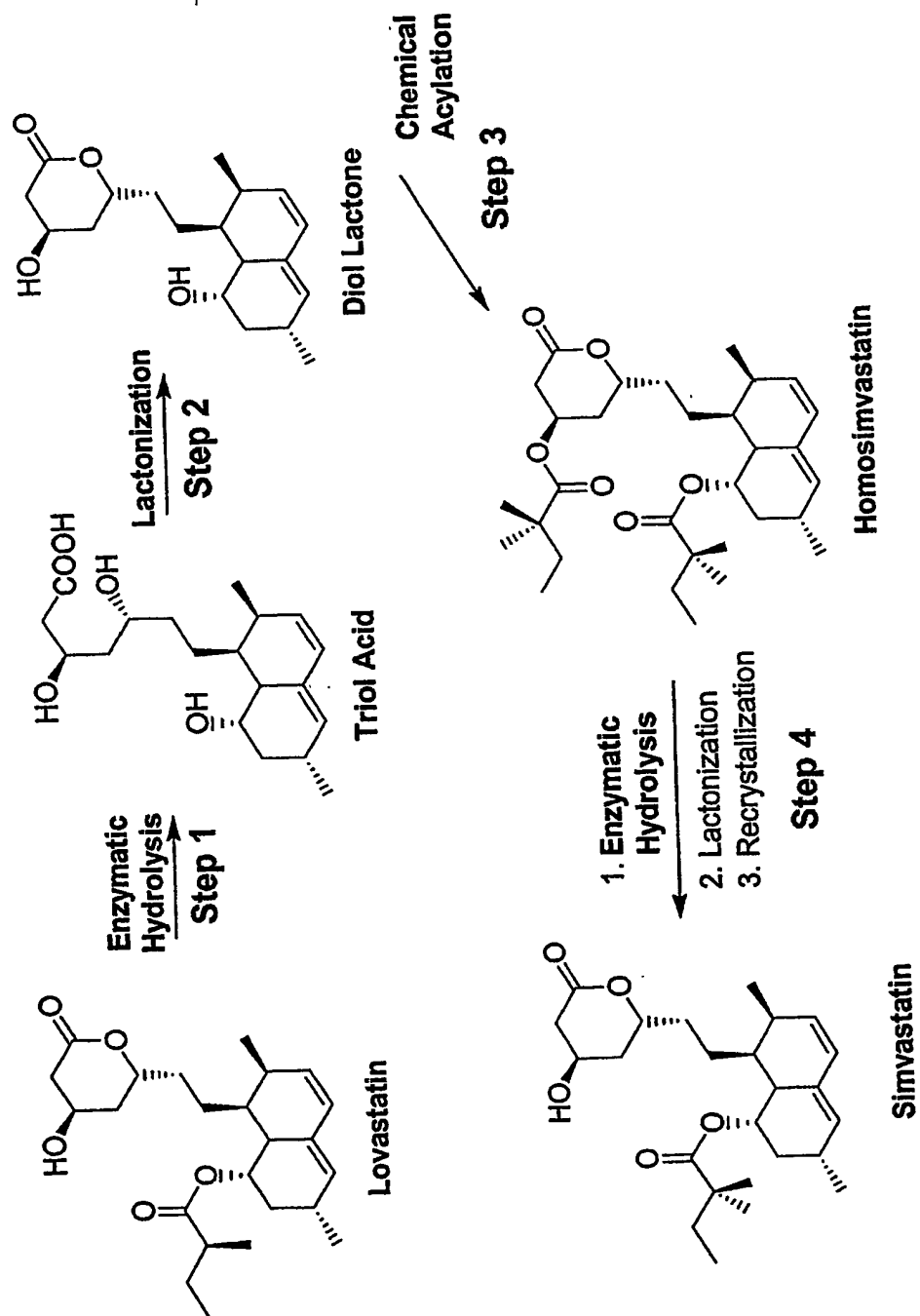


Figure 39

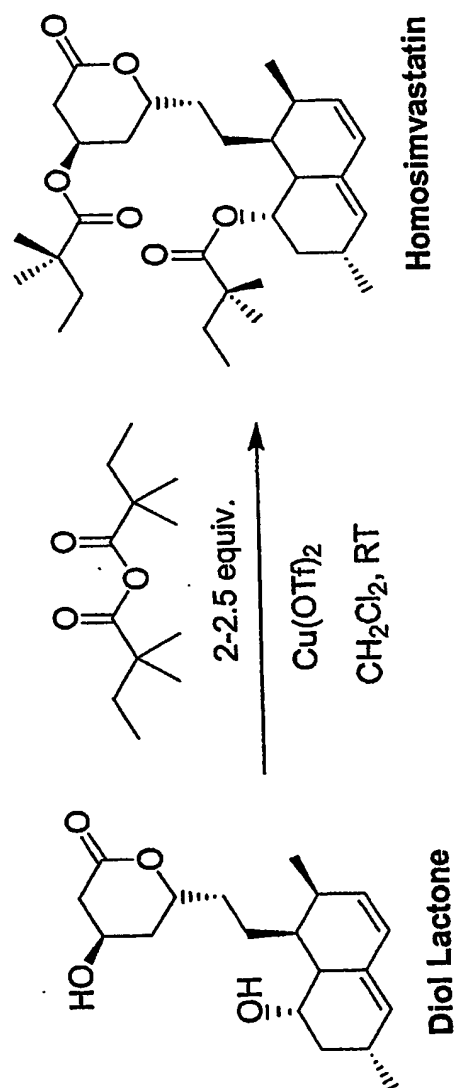


Figure 40

Figure 40A

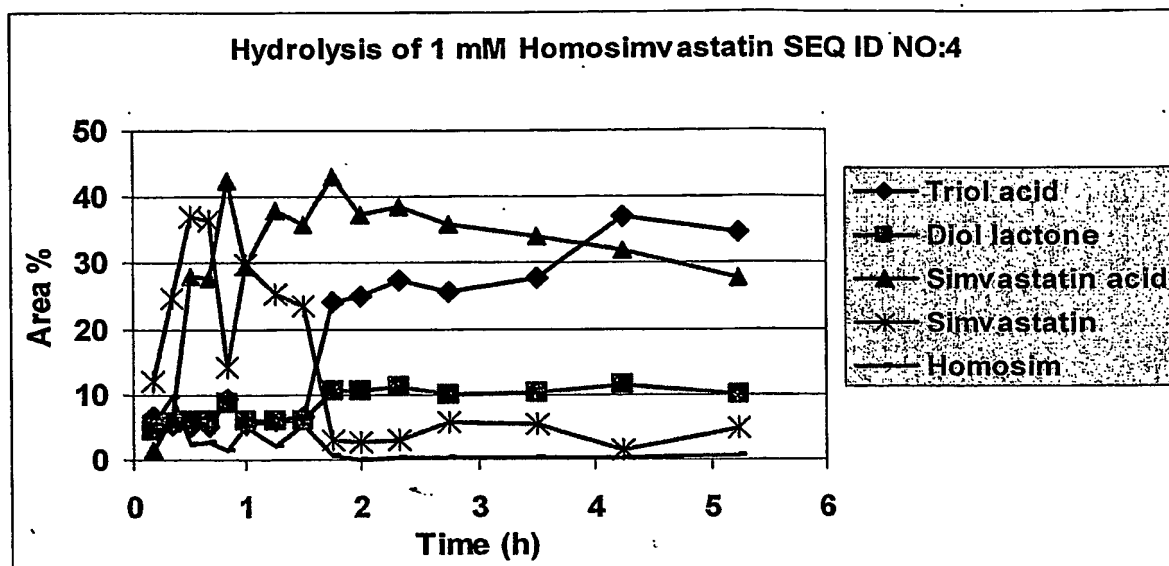
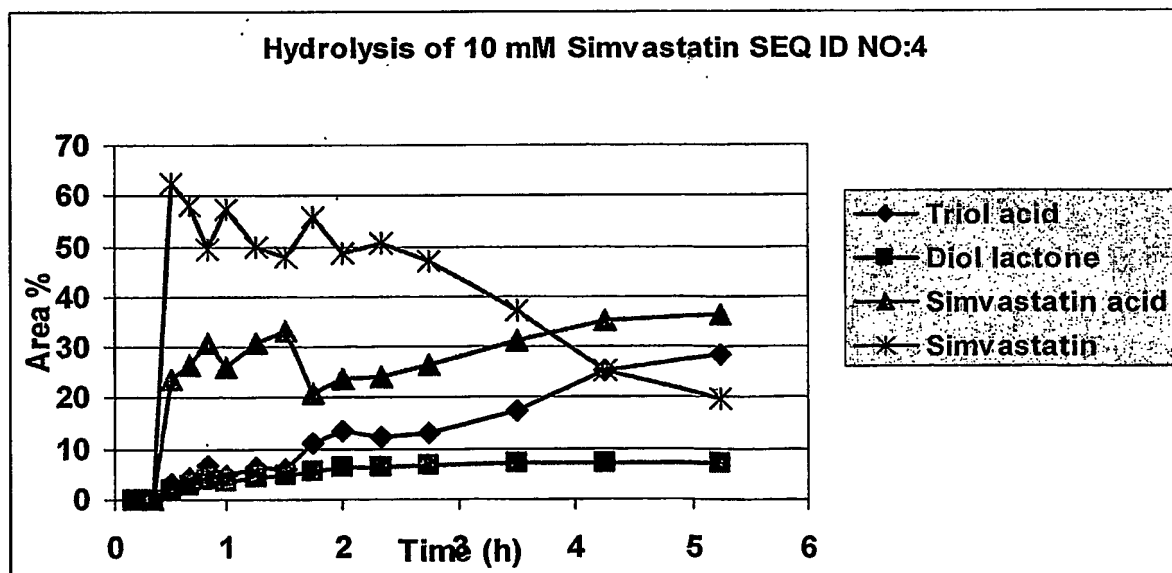


Figure 40B



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